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Effects of Suspended Solids on Selected Estuarine Plankton

by

J.A. Sherk, Jr., J.M. O'Connor, and D.A. Neumann

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A 3-year laboratory study identified biological components of selected populations of estuarine organisms which were most sensitive to the effects of particle size and concentration of (a) suspended mineral solids similar in size to sediments likely to be found in, or added to, estuarine systems in concentrations typically found during flooding, dredging, and disposal of dredged material, and (b) natural sediments in identical experiments.

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20. Abstract. (Continued)

Carbon assimilation by four species of phytoplankton was significantly reduced by the light attenuating properties of fine silicon dioxide suspensions. Ingestion of radioactively tagged food cells by two species of calanoid copepods was significantly reduced during exposure to suspensions of Fuller's earth, fine silicon dioxide, and natural Patuxent River silt. This report provides base-line data for preproject decisionmaking based on concentration effects of different suspended sediments on selected typical estuarine plankton.

PREFACE

This report is published to provide coastal engineers with a study of the effects of suspended sediment on estuarine organisms, in the continuing research on the ecological effects of engineering activities. It presents the results of a 3-year laboratory study on the subject. The work was carried out under the coastal ecology research program of the U.S. Army Coastal Engineering Research Center (CERC).

This report is published, with only minor editing, as received from the contractor; results and conclusions are those of the authors and are not necessarily accepted by CERC or the Corps of Engineers.

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Comments on this publication are invited.

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JAMES L. TRAYERS
Colonel, Corps of Engineers
Commander and Director

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EFFECTS OF SUSPENDED SOLIDS ON SELECTED ESTUARINE PLANKTON

by

J.A. Sherk, Jr., J.M. O'Connor,
and *D.A. Neumann*

I. EFFECTS ON SUSPENDED SOLIDS ON CARBON ASSIMILATION OF SELECTED SPECIES OF ESTUARINE PHYTOPLANKTON

1. Introduction.

Phytoplankton growth is usually measured in terms of photosynthesis (carbon uptake and oxygen evolution). This growth responds in a complex way to various single or combined factors. Some of the more important factors are nutrition, temperature, salinity, and light (Jitts, et al., 1964).

The release of silt, clay, and fine sand-sized particles (organic and inorganic) into the water column is a dominant feature of bay and estuarine areas (Sherk, 1971). These releases may be caused by beach erosion, river contribution, storm agitation, dredging, and disposal of dredged material. An important effect of the resulting suspended load is reduced light penetration (by absorption, Raleigh small and large particle scatter), which limits the depth of water in which light intensity is sufficient for the rate of phytoplanktonic photosynthesis to exceed the respiration rate.

The experimental work investigating the effects of increasing concentrations of extremely fine silicon dioxide particles on the carbon assimilation of various monospecific cultures of phytoplankton grown at estuarine salinities is presented in this section.

2. Materials and Methods.

a. Culture. Cultures of phytoplankton were obtained from the Woods Hole Oceanographic Institution (WHOI) as follows:

Name	WHOI clone symbol
Monochrysis lutheri	Mono
Nannochloris sp.	GSB Nanno
Stichococcus sp.	GSB Sticho
Chlorella sp.	0-10

These cultures were grown in Guillard's f/2 medium (Guillard and Ryther, 1962) made with autoclaved (15 pounds per square inch; 30 minutes) Patuxent River water which was previously filtered through sintered Whatman GFC glass-fiber filters.

Stock cultures of each species were maintained in 50 milliliters of the f/2 medium (salinity range 5.5 to 7.5 parts per thousand) contained in 125-milliliter Erlenmeyer flasks either plugged with sterile cotton wads or tightly covered with aluminum foil. Cultures (1 milliliter of stock) were transferred biweekly into fresh sterile (autoclaved) medium. No attempt was made to maintain axenic (bacteria-free) cultures. The cultures were checked periodically to (a) detect possible contamination by other species of phytoplankton, and (b) monitor growth rate of the stock (cells were counted at 100X on an improved Neubauer hemacytometer). Motile forms were fixed before counting with 6.5 percent glutaraldehyde (pH = 7.4) buffered with sodium cacodylate (0.14 molar).

Cultures were grown at 20 ± 0.5° Celsius under continuous cool white fluorescent illumination (0.8×10^4 ergs cm^{-2} sec^{-1}) in a constant temperature incubator. Under these conditions, an exponential growth rate (Sorokin, 1969) of 0.35 to 0.40 day^{-1} could be maintained for these species at cell concentrations between 5×10^5 and 5×10^6 cells ml^{-1}.

Before starting an experiment, approximately 10 milliliters (depending on cell concentration) of stock culture in exponential growth phase were transferred into 1.5 liters of fresh, sterile (autoclaved) f/2 medium (enriched, filtered Patuxent River water) in sterile Erlenmeyer flasks (2-liter capacity). The increase in cell numbers under culture light intensity was monitored until a cell concentration of approximately 50,000 cells ml^{-1} was attained. The experimental run was then conducted with minimum dilution of these cells by fresh medium. This cell concentration (50,000 cells ml^{-1}) may be representative of natural concentrations in certain areas of the Chesapeake Bay system (Flemer, 1972, personal communication). Carbon-12 concentrations during experimental work and in stock cultures were monitored by the method of Karlgren (1962) reported in Sherk (1969). Dissolved inorganic carbon concentrations were maintained at 15 to 20 milligrams $C\ l^{-1}$ in cultures and experimental media.

b. Sediments. A graded series of concentrations of silicon dioxide (Fisher No. S-153; see App.) was used for all assimilation experiments.

c. Determination of the Saturation Curves. Saturation curves were determined for phytoplanktonic photosynthesis under the illumination of six 40-watt cool white fluorescent lamps. The light was passed through Pyrex infrared reflecting glass (Kaufmann Glass Company, Wilmington, Delaware) before reaching the Plexiglass top of a constant temperature shaking-type incubator. The light intensity recorded inside the incubator at the incubation bottle location was 1.45×10^4 ergs cm^{-2} sec^{-1}. All measurements of light intensity were made with a Y.S.I. Model 65A radiometer.

Two sets of six ground glass-stoppered bottles (130 ± 1.5 milliliters capacity) were used for each determination. These bottles were previously washed in 0.01 N H_2SO_4, rinsed twice with deionized glass-distilled water, and rinsed twice with filtered, sterile (autoclaved) Patuxent River water enriched with f/2 medium. Each bottle was filled with approximately 5 x 10^4 cells (differences between bottles could not be distinguished from counting error, $p<0.1$).

Replicate pair of bottles was wrapped in black plastic window screen to attenuate light. The first pair was left unwrapped; the second pair was wrapped in one layer of screen; and the third, fourth, fifth, and sixth pairs were wrapped in 2, 3, 4, and 8 layers of screen, respectively.

After the bottles were filled with cells and placed within the screen layers, 1.0 microcurie $NaHC^{14}O_3$ (1-milliliter solution adjusted with NaOH to pH 9.5 in distilled water) was injected into each bottle with a Hamilton gas-tight syringe. For work with *M. lutheri*, 10.0 microcurie $NaHC^{14}O_3$ was injected into each bottle. The syringe was rinsed once with water from each bottle after initial injection. The bottles were stoppered and incubated in the constant temperature (20° Celsius) shaking (2 cycles per second) incubator for 4 hours under a light intensity of 1.45 x 10^4 ergs cm^{-2} sec^{-1}.

After incubation, the replicate pairs of bottles were removed from the incubator. Carbon assimilation of the phytoplankton was determined immediately by the acidification and bubbling technique of Schindler, Schmidt, and Reid (1972). Two 20-milliliter samples were withdrawn from each incubation bottle and placed into two short Allihn tubes (30-milliliter capacity) each containing 0.5 ml 0.1 N HCL to ensure complete stripping of dissolved inorganic carbon. Air was bubbled through the tubes for 20 minutes to remove the dissolved inorganic carbon. After bubbling, two 2-milliliter samples were withdrawn by pipet from each tube and placed into liquid scintillation vials. Fifteen milliliters of Bray's (1960) dioxane-base scintillation fluid were added to each vial.

All samples were counted at 9° Celsius using a Packard Tricard 3320 liquid scintillation spectrometer. After background subtraction, sample counts were corrected for quenching by an external standard quench curve constructed from counts of standard toluene carbon-14 with increasing amounts (0 to 400 microliters) of chloroform in 15 milliliters of Bray's solution. Counting efficiency of most of the samples was approximately 85 percent. Sample counts were averaged for each set of screens. Results are reported as gross counts per minute resulting from the carbon-14 assimilated per milliliter of sample per hour of incubation (cpm ml^{-1} hr^{-1}).

d. Determination of Suspended Solids Effects. Two sets of assimilation experiments were conducted with each of the four phytoplankton species exposed to graded concentrations of silicon dioxide. Both the assimilation effects and light saturation curves were determined for each species on the same day. *Monochrysis lutheri* was exposed to increasing concentrations (100, 250, 500, 1,000, and 2,250 milligrams l^{-1}) of the silicon dioxide (SiO_2) particles with a median size approximately 17 micrometers. The other three species were exposed to graded concentrations (50, 100, 500, and 1,000 milligrams l^{-1}) of the SiO_2 particles with a median size of 6.2 micrometers (see App.).

A slurry of SiO_2 particles was made up with filtered, sterile (autoclaved), enriched (f/2 medium) Patuxent River water before the start of each experiment. A magnetic stirring device was used to maintain the suspension. Concentration of particles by weight (milligrams l^{-1}) was determined by the dry weight difference between the average of three oven-dried aliquots (5 milliliters each) of the slurry and the average of three aliquots (5 milliliters each) of the enriched river water (salt correction).

Fifteen glass-stoppered bottles, rinsed and washed as in the saturation curve determinations, were used for each experiment. Of these, five replicate pairs were light bottles. Each of these pairs had a corresponding dark bottle which had been wrapped with two layers of heavy gage aluminum foil. All bottles were filled with cells (approximately 50,000 cells ml^{-1}) from the same culture flasks. Small amounts of the SiO_2 slurry were measured by volume (Oxford precision micropipet) and injected into the bottles (two light and one dark bottle received the same concentration) to make up the series of increasing SiO_2 concentrations. The amount of slurry injected never exceeded 0.5 percent of bottle volume (130 milliliters). After approximately 1.0 microcurie $NaHC^{14}O_3$ was injected into each bottle with a gas-tight syringe dark bottles were completely wrapped and all bottles were stoppered.

The rest of the procedure (incubation and isotope counting) was similar to that of the saturation curve determinations with these exceptions:

(1) Dark bottle counts (dark carbon fixation) were subtracted from the average of the light bottle samples after background subtraction and quench correction.

(2) Results are reported as net counts per minute assimilated per milliliter per hour (cpm $ml^{-1}hr^{-1}$) during exposure to increasing concentrations of SiO_2.

(3) The reduction in carbon uptake (expressed as percent of control) is reported at each concentration of SiO_2.

3. Results and Interpretation.

a. Saturation Curves. The spectral quality of light is important in studies of photosynthesis (see action spectra in Jitts, et al., 1964); however, there is no typical submarine spectral distribution because of the interactions of depth, the light attenuating properties of seawater, and the conditions of the sky. Light of wavelengths greater than 6,500 angstroms or less than 4,000 angstroms may be missing even in moderately clear coastal waters (Jitts, et al., 1964). No form of submarine light resembles the spectral distribution of unfiltered sunlight or the illumination from cool white fluorescent lamps.

Light attenuation by the use of successive layers of black plastic screen provides a rough geometrical decrease in intensity. One screen layer approximately reduced the intensity to 59 percent of the experimental incident radiation with no screen, two layers approximately attenuated the intensity to 40 percent, three layers to 28 percent, four layers to 17 percent, and eight layers to 7 percent.

The low light intensities used in culturing the phytoplankton experimentally are thought to be typical of upper to middle turbid areas of Chesapeake Bay tributaries at depths greater than 0.5 meter. These intensities were calculated roughly from our own water surface insolation measurements with the Y.S.I. radiometer and Flemer's extinction coefficients; conversion factors were according to Strickland (1958).

Under the experimental conditions, a light intensity of 1.45 x 10^4 ergs provided nearly optimal photosynthesis by *M. lutheri* (Fig. 1). This value is approximately 41 percent of that reported by Craigie (1969) for this species. Our value is approximately 14 percent of that reported by Jitts, et al., (1964) for optimal growth of this species at 19° Celsius, although their preconditioning intensity was roughly 4.55 x 10^4 ergs cm^{-2} sec^{-1} versus 0.8 x 10^4 ergs cm^{-2} sec^{-1} in our project. They also used enriched synthetic seawater with a salinity of about 29 parts per thousand compared to our filtered, natural Patuxent River water enriched with f/2 medium (salinity 5.5 to 7.5 parts per thousand). These data show that *M. lutheri* can be acclimated to grow optimally under different laboratory conditions.

The saturation curves for *Chlorella* sp. (Fig. 2) and *Nannochloris* sp. (Fig. 3) indicate that these species were close to light saturation also. *Stichococcus* sp. is farthest from the light saturation plateau (Fig. 4).

b. Effects of Suspended Solids. Carbon uptake of *M. lutheri* (Fig. 5) progressively decreased with increasing concentrations of SiO_2. Nearly an 80-percent reduction in uptake occurred at 2,250 milligrams l^{-1} SiO_2 (median size = 17 micrometers). *Chlorella* sp. (Fig. 6) showed a 90-percent decrease in carbon uptake at concentrations of 1,000 milligrams l^{-1} of <15 micrometers SiO_2 (median size = 6.2 micrometers). Practically the same magnitude of reduction in carbon uptake occurred at concentrations of 1,000 milligrams l^{-1} of 15

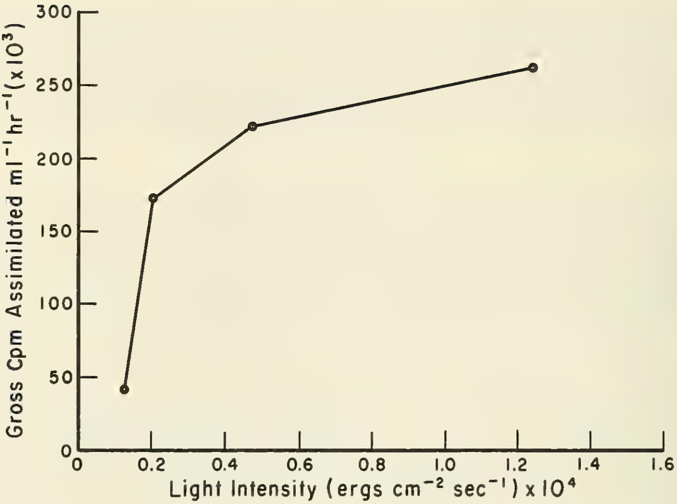

Figure 1. Saturation curve of photosynthesis for *Monochrysis lutheri* exposed to cool white illumination.

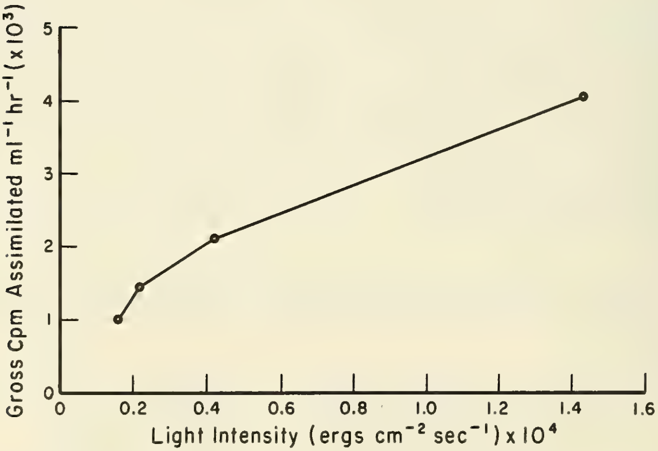

Figure 2. Saturation curve of photosynthesis for *Chlorella* sp. (cultured under 0.8 x 10^4 ergs cm^{-2} sec^{-1}) exposed to cool white fluorescent illumination.

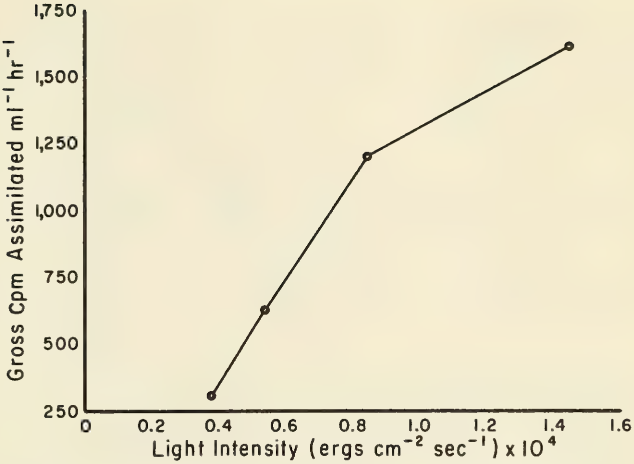

Figure 3. Saturation curve of photosynthesis for *Nannochloris* sp. (cultured under 0.8 x 10^4 ergs cm^{-2} sec^{-1}) exposed to cool white fluorescent illumination.

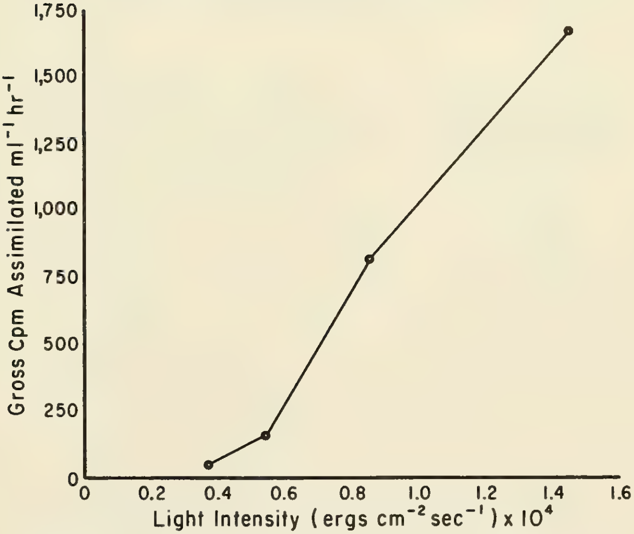

Figure 4. Saturation curve of photosynthesis for *Stichococcus* sp. (cultured at 0.8 x 10^4 ergs cm^{-2} sec^{-1}) exposed to cool white fluorescent illumination.

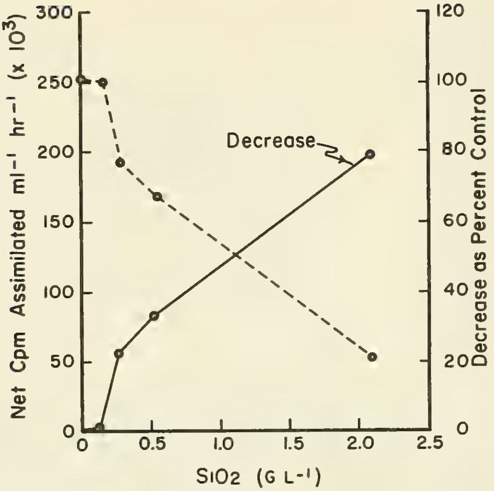

Figure 5. The effect of increasing concentrations of silicon dioxide on carbon assimilation of *Monochrysis lutheri*.

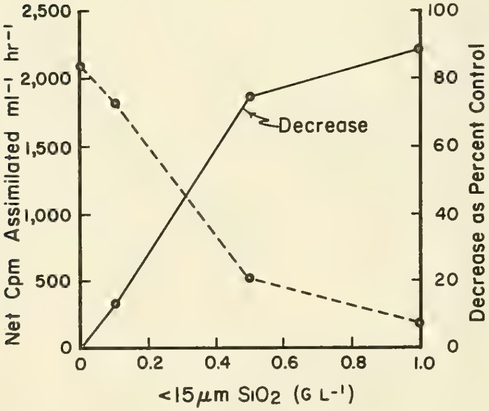

Figure 6. The effect of increasing concentrations of silicon dioxide (all particles <15 micrometers) on carbon assimilation of *Chlorella* sp.

micrometers SiO_2 for the other two species (Figs. 7 and 8). The carbon uptake curves for *M. lutheri*, *Chlorella* sp., and *Nannochloris* sp. were practically the same shape (Fig. 8) and were not linear. The peculiar shape of the *Stichococcus* sp. curve (Fig. 8) supports the idea that photosynthesis for this species was light-limited even before exposure to concentrations of solids.

These changes in carbon uptake support the relationships reported by Biggs (1970) for increasing suspended particle concentration and decreasing transmission of light in water determined with increasing concentrations of kaolinite (median size = 4.0 micrometers). The kaolinite concentrations decreased the light less than the same concentrations of particles occurring in a natural water sample (median size = 1.9 micrometers).

The effects of SiO_2 on *M. lutheri* were less than for either *Nannochloris* sp. or *Chlorella* sp. (Figs. 5, 6, and 7). However, *M. lutheri* was exposed to a median particle size of 17 micrometers, as opposed to 6.2 micrometers for the other two species. Evidently, greater carbon uptake by *M. lutheri* was partly due to increased light transmission through the larger particles, although concentrations were identical for all species. A concentration of 2,250 milligrams l^{-1} SiO_2 caused an 80-percent reduction in carbon uptake by *M. lutheri*. At 1,000 milligrams l^{-1} this reduction was approximately 50 percent. For the other three test species in the finer SiO_2, at 1,000 milligrams l^{-1}, the the reduction was almost 90 percent.

The motility of *M. lutheri* must also be considered. As light intensity attenuated, this species could have moved toward the sides of the incubation bottles, i.e., nearer to the source of incident light. This movement might have contributed substantially to improved carbon uptake, although it was still much reduced at higher concentrations of SiO_2.

Regardless of particle-size distribution relationships, the reductions in carbon uptake demonstrated for concentrations between 100 and 500 milligrams l^{-1} are biologically significant with respect to the reduced energy available to filter-feeding organisms (see Section II). Concentrations of particles with similar size distributions are typical of those occurring during dredging and disposal operations (May, 1973; Masch and Espey, 1967) (see Table A-3, App.), resuspensions during storms, or as normal "high" background values in Galveston Bay (Masch and Espey, 1967) and Louisiana marshes (Mackin, 1961).

An additional factor associated with dredging may be the release of organic matter and minerals into the water column which may be either beneficial and stimulate growth (Odum, 1973; Flemer, 1970) or detrimental. The amount of material released (May, 1973) and the dilution rate is unknown. Nutrients, heavy metals, and pesticides can be released into biological pathways and be magnified in the trophic

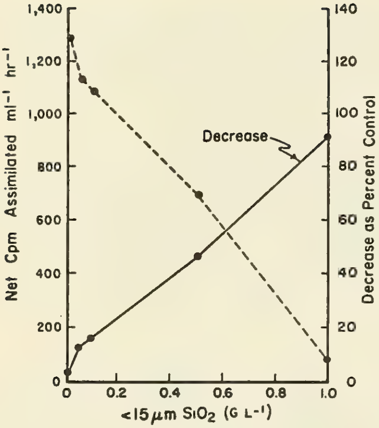

Figure 7. The effect of increasing concentrations of silicon dioxide (all particles <15 micrometers) on carbon assimilation of *Nannochloris* sp.

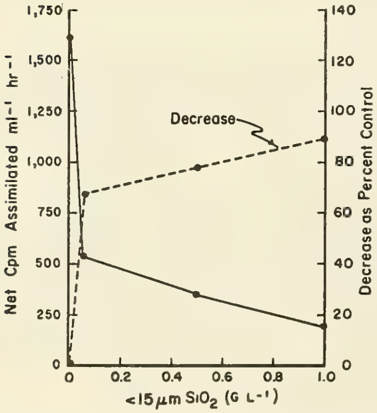

Figure 8. The effect of increasing concentrations of silicon dioxide (all particles <15 micrometers) on carbon assimilation of *Stichococcus* sp.

structure of estuarine systems. No interference was anticipated or evident in these carbon uptake studies since we used relatively "inert" SiO_2 particles (see App.). Nevertheless, future work should research the effects of these factors in addition to the effect of light attenuation on productivity of phytoplankton species, groups of species, and natural populations.

II. EFFECTS OF SUSPENDED SEDIMENTS ON FEEDING ACTIVITY OF THE COPEPODS *EURYTEMORA AFFINIS* AND *ACARTIA TONSA*

1. Introduction.

Suspension feeding estuarine organisms are exposed to high suspended sediment concentrations at times. Mechanical or abrasive action of suspended particles causes gill clogging, impairment of respiratory and excretory functioning, and reduced feeding effectiveness. In strongly agitated water a substantial part of the suspended materials consists of fine sand, silt, and clay-sized particles which have been resuspended from the bottom. Considerable quantities of these inorganic (mineral) solids can enter the gut of filter-feeding aquatic organisms with particulate food.

This section presents results of studies on the interference of suspended mineral solids and resuspended natural sediments with the feeding activity of the calanoid copepods *Eurytemora affinis* and *Acartia tonsa* (typical Chesapeake Bay zooplankton).

2. Materials and Methods.

a. Zooplankton Cultures. Cultures of *E. affinis* and *A. tonsa* were made available for this project by Dr. Don Heinle and Mr. Joseph Ustach, Chesapeake Biological Laboratory, Solomons, Maryland. Cultures were held in a constant temperature incubator at 20° Celsius under constant cool white fluorescent illumination (0.8×10^4 ergs cm^{-2} sec^{-1}) in Erlenmeyer flasks (2-liter capacity) containing 2 liters of Patuxent River water (salinity range 5.5 to 7.5 parts per thousand) which was filtered previously through sintered Whatman GFC glass fiber-filter pads.

The zooplankters initially were fed a mixed diet of the motile chrysophyte *M. lutheri* and the bacillariophyte *Phaeodactylum tricornutum*. Cell concentrations of these phytoplankters were maintained within a range of 5×10^4 to 1×10^5 cells ml^{-1} in the zooplankton cultures. Erratic feeding rates during initial feeding experiments coupled with the "blooming" tendency of the *P. tricornutum* in the zooplankton cultures caused the diet of these cultures to be limited to *M. lutheri*, especially during the days preceding a feeding rate experiment.

Every third or fourth day, one-third to one-half of the water in each culture flask was replaced with new glass fiber-filtered Patuxent River water of the same salinity. At the same time additional phytoplankton cells were added to the zooplankton culture flasks to make up for (a) the amounts removed and dilution of the remaining cells with new filtered Patuxent water, and (b) the amounts grazed by the growing culture.

Efforts were made to keep each culture in a state of rapid growth (exponential growth phase) so a constant supply of adults of both species (both sexes) would be available for experimental work. *Acartia tonsa* tended towards cannibalism when the adult population in each flask exceeded 20 to 30 individuals. As a result, the culture population would boom and crash unexpectedly. This necessitated maintenance of this species in many culture flasks, and required close monitoring of adult populations, available algal food supply, and water quality.

b. Phytoplankton Cultures. Cultures of the phytoplankters *M. lutheri* and *P. tricornutum* were maintained in an exponential growth phase in filtered sterile (autoclaved) Patuxent River water enriched with f/2 medium as described in the previous section.

c. Sediments. Some physical and chemical characteristics of sediment types used for experimental work in this section are presented in the Appendix. The sediment types and concentrations used for each zooplankton species were as follows:

Eurytemora affinis

(1) SiO_2 (50, 100, 1,000, 10,000 milligrams l^{-1}).

(2) <15 micrometers SiO_2 (50, 100, 500, 1,000 milligrams l^{-1}).

(3) Fuller's earth (50, 100, 500, 1,000 milligrams l^{-1}).

(4) Natural sediment (50, 100, 250, 500, 1,000 milligrams l^{-1}).

Acartia tonsa

(1) <15 micrometers SiO_2 (50, 100, 500, 1,000 milligrams l^{-1}).

(2) Fuller's earth (50, 100, 500, 1,000 milligrams l^{-1})

(3) Natural sediment (50, 100, 500, 1,000 milligrams l^{-1}).

d. Determination of Time to Maximum Ingestion. Feeding experiments were conducted using *M. lutheri* labeled with carbon-14. Cell motility greatly reduced the need for shaking the incubation bottles during experiments. These studies measured the radioactive uptake by zooplankton that were not yet producing radioactive fecal material. The method is outlined in Rigler (1971) and was introduced by Nauwerck (1959) from a slightly modified technique originally used by Marshal and Orr (1955). This method eliminates three problems usually associated with feeding rate determinations:

(1) It is suitable for measurement of feeding rates of organisms that do not produce coherent fecal pellets.

(2) It eliminates the need for estimates of the leaching of tracer carbon out of fecal pellets.

(3) It reduces the error associated with excretion of tracer carbon (some ingested food cells remain undigested at the end of the short-feeding period).

Two ground glass-stoppered bottles (130-milliliter capacity) containing high cell concentrations of actively growing *M. lutheri* were each injected with approximately 10 microcurie $NaHC^{14}O_3$." These cells were incubated under cool white fluorescent illumination at 20° Celsius for 4 hours. After incubation the cells were centrifuged at 6,000 revolutions per minute for 10 minutes. Supernatant water was decanted and the cells were resuspended in enriched, sterile, filtered Patuxent River water (Section I). The cells were then recentrifuged, the supernatant decanted, and the cells suspended a second time. This procedure removed most of the unassimilated dissolved inorganic carbon-14 and excreted fixed carbon compounds which had accumulated in the bottles during the incubation period. After the second resuspension, cell concentration was counted at 100X on an improved Neubauer hemacytometer. The radioactivity of a 10-milliliter aliquot of this suspension was determined by the acidification and bubbling procedure of Schindler, Schmidt, and Reid (1972) outlined in Section I.

During the phytoplankton incubation period, replicate pairs of ground glass-stoppered bottles (130-milliliter capacity) to contain the zooplankton during the feeding experiment were prepared. Each pair of bottles represented a specific time series interval of feeding activity for zooplankton exposed to radioactive phytoplankton cells (food). These intervals were 5, 10, 15, 20, 30, 45, and 60 minutes or longer depending upon the number of adults of either species available for experimental use. Each bottle was rinsed twice with 0.01 normal H_2SO_4 to remove any carbon-14 from previous experiments, rinsed twice with deionized glass-distilled water, rinsed twice with filtered (Whatman GFC glass-fiber filters) Patuxent River water, and filled with 50 milliliters of the filtered river water (salinity range 5.5 to 7.5 parts per thousand).

Adult zooplankters (both sexes) of the species being investigated were captured individually from the culture flasks in a minimum amount of water (usually less than 5 milliliters per individual). The capturing tool was a glass tube fitted with a rubber-suction bulb at one end for drawing the copepod into the tube. Ten copepods were placed in each bottle. Bottle volume was adjusted to 125 milliliters with water from the zooplankton culture flasks containing the phytoplankter *M. lutheri*. In this way, phytoplankton cell concentrations (not radioactive) in the bottles varied from 5,000 to 10,000 cells ml^{-1}. Zooplankters were allowed to feed on these cells for 0.5 to 1 hour before the start of the experiment. During this time they also became somewhat acclimated to the environment of the bottle.

The feeding experiment was initiated by injecting a volume of radioactive *M. lutheri* cell suspension into one bottle of a replicate pair of bottles containing 10 zooplankters each. The other bottle in this pair received an identical volume of the radioactive cells 5 minutes later. This cell suspension volume never exceeded 1 percent (1.25 milliliters) of bottle volume, but it was sufficient to provide a final radioactive cell concentration of close to 50,000 cells ml^{-1}. Experiments were conducted at 20 ± 2° Celsius under laboratory cool white fluorescent illumination (approximate intensity = 0.2×10^4 ergs $cm^{-2}sec^{-1}$). Feeding activity was terminated by pouring the contents of each bottle onto a stainless steel (type 304) wire sieve (325 mesh). The bottles were rinsed with 50 milliliters of Whatman GFC filtered Patuxent River water. This water was then poured through the sieve. Preliminary studies showed that this 50-milliliter rinse effectively washed away sorbed carbon-14 and any phytoplankton cells adhering to the zooplankters which were trapped on the stainless steel mesh. Immediately after rinsing, the sieve was placed under a dissecting microscope and the live zooplankters were removed individually by grasping an antenna with fine-pointed forceps. Each organism was placed into a separate liquid scintillation vial containing 1 milliliter of Soluene-100 (Packard Instrument Company, Downers Grove, Illinois), a rapidly acting tissue solubilizer (quarternary amine). The vials were capped and digestion was allowed to proceed for at least 2 hours at room temperature. With practice all zooplankters (10 under optimal experimental conditions) could be efficiently removed from the sieve and placed into liquid scintillation vials within 2 minutes of feeding termination.

The zooplankters in successive replicate pairs of bottles representing longer feeding time intervals were handled in exactly the same way (5-minute lag between bottles of a replicate pair).

At the end of the digestion period, 15 milliliters of acidified Instagel were added to each vial. Acidified Instagel was prepared as a 1:9 (volume to volume) solution of 0.5 N HCl and Instagel (Packard Instrument Company, Downers Grove, Illinois). The vials were placed into a Packard Tricarb (Model 3320) liquid scintillation spectrometer and counted at 9° Celsius after allowing 24 hours for reduction of chemiluminescence, an undesirable feature of this counting fluid formulation. After background subtraction, counts were corrected for quenching by an external standard quench curve constructed from counts of standard toluene carbon-14 with increasing amounts (0 to 400 microliters) of chloroform in a solution of 15 milliliters of acidified Instagel and 1 milliliter of Soluene-100. Counting efficiency of most samples was approximately 88 percent.

Results are graphed as average carbon uptake (counts min^{-1} hr^{-1}) of the 20 zooplankters in the paired replicate bottles (10 from each bottle) plotted against time from initial feeding.

e. Determination of the Effects of Increasing Concentrations of Different Sediment Types on Zooplankton Feeding Activity at Time of Maximum Ingestion. Maximum ingestion (maximum uptake of carbon-14) of radioactive *M. lutheri* cells (50,000 cells ml^{-1}) at 20 ± 2° Celsius under approximately 0.2×10^4 ergs cm^{-2} sec^{-1} light intensity in a salinity range of 5.5 to 7.5 parts per thousand was 5 minutes for adult *A. tonsa* and 10 minutes for adult *Eurytemora affinis*.

Slurries of different sediment types were prepared and maintained as outlined in Section I, except that the filtered Patuxent River was not enriched with f/2 medium. Slurries of natural sediment were aerated to a light tan color before use in these experiments. This reduced the biochemical oxygen demand (BOD).

Phytoplankton, zooplankton, and scintillation vials were prepared as in the preceding section with the following procedural changes:

(1) For *E. affinis*, replicated experimental sets with series of increasing concentrations were conducted with the two different size distributions of SiO_2 (see App.). Only one experimental run with Fuller's earth was made, but all bottles were replicated. Two experimental runs were made with natural sediment and only the controls were replicated.

(2) For *A. tonsa*, two experimental runs with replicated controls were made with Fuller's earth and with SiO_2 (15 micrometers). Only one experimental run was made with natural sediment due to a shortage of adults.

(3) The time course of the feeding experiments with increasing concentrations of each sediment type was 5 minutes for *A. tonsa* and 10 minutes for *E. affinis*.

(4) After the zooplankters were placed into the bottles and acclimated for 0.5 to 1 hour as before, appropriate amounts (by volume) of sediment slurry were added by precision micropipet to successive bottles (pairs in some cases) to create an increasing series of sediment concentrations. The amount

added for maximum sediment concentration did not exceed 1 percent of bottle volume. An appropriate volume of radioactive *M. lutheri* cells was added by micropipet, as before, to each bottle to provide a cell concentration of 50,000 cells ml^{-1}.

The natural sediments and mineral solids used were so fine-grained that no settling of particulate material occurred during the short time of the experiments. Therefore, bottles containing the zooplankton were not shaken.

Preliminary work showed that the natural sediments and mineral solids used did not sorb carbon-14 counts that were significantly higher than background.

Results are reported as the difference between the uptake in control and sediment treatments and are expressed as a percent of the control value. The differences between carbon uptake in controls and sediment treatments were tested for significance by Student's "t" test (Snedecor and Cochran, 1967). Values for individual zooplankters were coded and logarithmically transformed before testing was done.

f. Determination of the Effects of Suspended Patuxent River Sediment (500 milligrams l^{-1}) on Zooplankton Feeding Activity with Increasing Time of Exposure. The procedure for this determination was identical with that used for the determination of time to maximum ingestion with these exceptions:

(1) Replicate pairs of bottles were not used because of the extensive time series (5, 10, 15, 30, 45, and 60 minutes; 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, and 4 hours). Instead, two sets of bottles (one control and one sediment treatment), each containing 10 adult zooplankters were used at each interval in the series.

(2) Sufficient natural sediment slurry was added to each sediment treatment bottle to make the concentration 500 milligrams l^{-1}. Radioactive *M. lutheri* cells were pipetted simultaneously into a control bottle and its corresponding sediment treatment bottle for a specific feeding time interval. This procedure was continued throughout the time series of control and treatment bottle pairs. Bottle pairs for the long-time intervals were inverted every 15 minutes to maintain the particles in suspension.

Results are reported as cells ingested per organism per day (cells $org^{-1} day^{-1}$). This daily rate (f) was calculated from the formula:

$$f = C(R_2 \times 24)/(R_f \times t),$$

where

R_2 = average activity (counts per minute) for zooplankters exposed to the sediment concentration for a given time,

R_f = average activity (counts per minute) of food cells (*M. lutheri*) in 1 milliliter of suspension,

t = feeding time in hours,

c = average concentration of food cells per milliliter over the feeding periods.

These values were plotted against feeding time. The differences between sediment treatment and control values at each feeding time were tested for significance with Student's "t" test after logarithmic transformation. Sample counts per minute were corrected by background subtraction and quench corrected from a curve constructed as before. This curve relates machine counting efficiency of standard toluene carbon-14 to counts of an external standard during addition of increasing amounts of a severe quenching agent (chloroform) to acidified Instagel-Soluene-100 liquid scintillation counting fluid.

3. Results and Interpretation.

a. Time to Maximum Ingestion. The shortest time in which a food particle could pass through the gut of *E. affinis* was demonstrated by a decrease in uptake of radioactivity for feeding periods in excess of 10 minutes (Fig. 9). These results are in good agreement with those of Heinle (1973, unpublished) who has noted formation of fecal pellets at 10 minutes after start of feeding for this species. Filtering rates for this species are lower than those reported for similar organisms by Anraku (1964). This may have been caused by a decrease in the size of new generations of adults as the age of the cultures increased.

The shortest time in which a food particle could pass through the gut of adult *A. tonsa* (Fig. 10) was 5 minutes.

There are two inflection points in the feeding rate curve for the first hour of feeding (Fig 9). The first inflection point occurred between 0 and 5 minutes (Fig. 10). These inflections are similar to those reported by Bourne (1959) for *Daphnia magna* feeding on 60,000 to 250,000 cells ml^{-1} of *Chlorella vulgaris* for 10 to 20 minutes and again for 40 to 55 minutes (Figs. 9 and 10), depending upon the cell concentration. Bourne's data demonstrated that the inflection of the carbon uptake curve at 40 to 55 minutes was associated with the onset of defecation following initial filling of the gut.

McMahon and Rigler (1963) found that the initial peak represented filling of the oral groove of this species. This collecting rate was higher by about 5 percent than the actual ingestion rate. However, the total time of these types of experiments, i.e., the time between

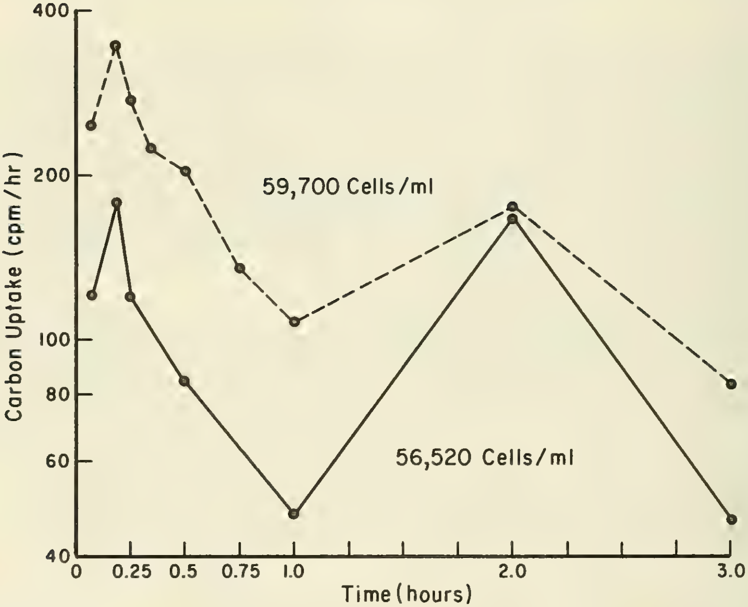

Figure 9. Ingestion of radiocarbon-labeled *Monochrysis lutheri* by adult *Eurytemora affinis* in water previously filtered through sintered Whatman GFC glass fiber filters.

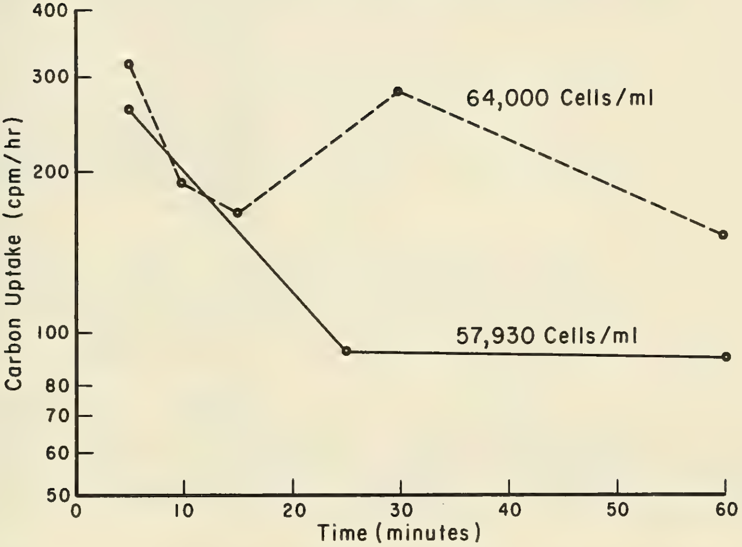

Figure 10. Ingestion of radiocarbon-labeled *Monochrysis lutheri* by *Acartia tonsa* (adults, mixed sexes) at 20° Celsius in water previously filtered through sintered Whatman GFC glass fiber filters.

initial exposure of zooplankton to radioactive food and their removal from the water for counting, must be less than the time for gut passage as sizable errors are introduced at longer intervals due to excretion and respiration of carbon-14 (Rigler, 1971). We chose the 5- and 10-minute intervals for the two copepods to coincide with Heinle's (1973, unpublished) observations of pelletformation in these two species.

b. Effects of Suspended Solids on Feeding Activity at Maximum Ingestion Time. Considerable quantities of inorganic material mixed with particulate food interferes with food ingestion by the copepods *E. affinis* and *A. tonsa*. Additions of two different particle-size distributions of fine sand, Fuller's earth, and Patuxent River silt to cell suspensions of heavily labeled ($NaHC^{14}O_3$) *M. lutheri* (50,000 cells ml^{-1}) significantly reduced the maximum ingestion rate of these cells by *E. affinis* at solids concentrations in excess of 250 milligrams l^{-1} (Fig. 11, Table 1). At 100 milligrams l^{-1} concentrations of Fuller's earth a significant increase occurred. Some nonsignificant reduction at this concentration occurred for other particle types.

The Patuxent River silt caused a greater reduction in ingestion of radiocarbon-labeled cells than other particle types over all concentrations tested except at the low concentration of 50 milligrams l^{-1} (Fig. 11, Table 1). The apparent enhancement effect of the silt at 100 milligrams l^{-1} was smaller than for all other particle types.

Acartia tonsa exhibited biologically significant reductions in ingestion at all concentrations of all particle types tested (Fig. 12, Table 2). The effects increased with increasing concentration. However, with this species the effect of Patuxent River silt was lower than Fuller's earth or SiO_2 particles (< 15 micrometers) except at the highest concentration (1,000 milligrams l^{-1}).

The differences in the shapes of the uptake curves may be related to the habitats of the two species. *Eurytemora affinis* typically occurs in upper, more turbid estuarine areas (Herman, Mihursky, and McErlean, 1968), where normally low concentrations of suspended solids may stimulate feeding. Low concentrations of suspended solids may indicate the presence of food to this species, and in turn stimulate the organism to begin feeding or to increase its feeding rate.

Stimulation of pumping activity in the American oyster at low particle concentrations (Loosanoff, 1961), increased activity of *Doliolida* and *Salpida* by the presence of suspended particles (Jorgensen, 1966), and tripling of the ingestion rate of algal cells by *Artemia* sp. upon the addition of fine sand to the cell suspension (Reeve, 1963) lend support to our observations of the response of *E. affinis*. This type of feeding response will increase the organism's chances for survival.

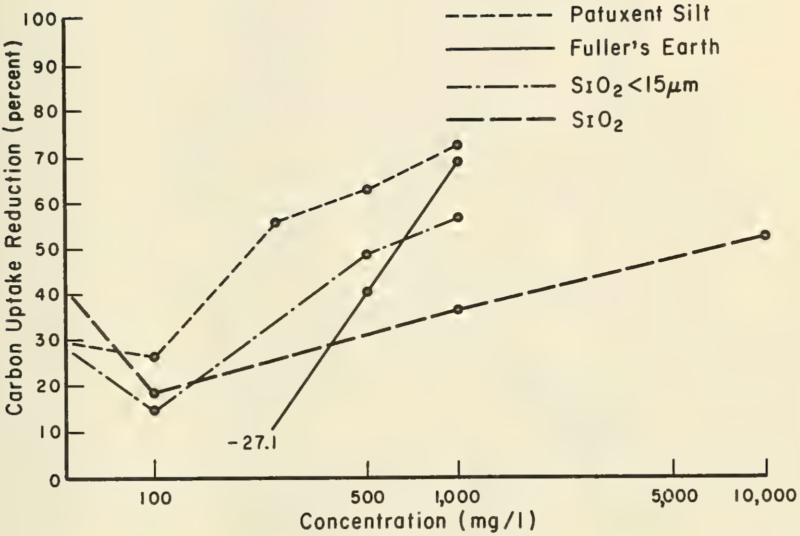

Figure 11. Reduction in maximum ingestion rate of adult *Eurytemora affinis* feeding on radiocarbon-labeled *Monochrysis lutheri* at increasing concentrations of Patuxent River silt, Fuller's earth, SiO_2, and <15 micrometers SiO_2.

Table 1. Effect of increasing concentrations of different sediment types on uptake of radioactively labeled *Monochrysis lutheri* by *Eurytemora affinis*.

Concentration (milligrams per liter)	SiO_2		<15 micrometers SiO_2		Fuller's earth		Natural sediment	
	$\bar{X} \pm S$ (N)	Reduction (percent of control)	$\bar{X} \pm S$ (N)	Reduction (percent of control)	$\bar{X} \pm S$ (N)	Reduction (percent of control)	$\bar{X} \pm S$ (N)	Reduction (percent) of control)
Run 1								
0	2.55 ± 1.51 (20)	0.0	46.24 ± 17.13 (18)	0.0	8.14 ± 2.55 (19)	0.0	47.03 ± 17.07 (20)	0.0
50	1.29 ± 0.59 (20)	49.4^1	32.77 ± 11.69 (19)	29.1^1	10.41 ± 2.01 (15)	-27.9^1	31.00 ± 9.93 (10)	34.1^1
100	2.07 ± 1.52 (19)	18.8^2	33.84 ± 10.4 (19)	26.8^3	10.35 ± 2.69 (17)	-27.1^1	36.50 ± 16.50 (8)	22.4^2
250	----------------	----	------------------	----	-----------------	----	13.57 ± 8.47 (8)	71.1^1
500	----------------	----	22.25 ± 11.84 (7)	51.9^1	4.75 ± 2.49 (20)	41.6^1	24.40 ± 10.02 (9)	48.1^1
1,000	1.52 ± 0.92 (19)	40.4^3	19.50 ± 6.87 (10)	57.8^1	2.54 ± 1.25 (20)	68.8^1	9.55 ± 8.94 (10)	79.7^1
10,000	1.34 ± 0.77 (17)	47.5^1	------------------	----	-----------------	----	------------------	----
Run 2								
0	4.10 ± 1.73 (20)	0.0	73.15 ± 32.09 (18)	0.0	-----------------	----	92.57 ± 48.33 (19)	0.0
50	2.60 ± 0.97 (20)	36.5^1	50.73 ± 16.16 (18)	30.7^3	-----------------	----	69.43 ± 23.93 (10)	25.0
100	3.30 ± 1.98 (19)	19.4^2	71.23 ± 18.46 (20)	2.6^2	-----------------	----	62.62 ± 21.72 (9)	32.4^3
250	----------------	----	------------------	----	-----------------	----	54.68 ± 19.30 (9)	40.9^3
500	----------------	----	38.71 ± 13.16 (20)	47.1^1	-----------------	----	21.24 ± 14.34 (9)	77.1^2
1,000	2.77 ± 1.25 (19)	32.4^1	33.24 ± 10.91 (18)	54.6^1	-----------------	----	33.09 ± 11.81 (10)	64.3^2
10,000	1.90 ± 1.55 (17)	53.7^2	------------------	----	-----------------	----	------------------	----

1. $p<0.01$.
2. Not significant.
3. $p<0.05$.

p = probability that means are the same.

NOTE: Mean values are ~~cycles~~ COUNTS per minute taken up in 10 minutes.

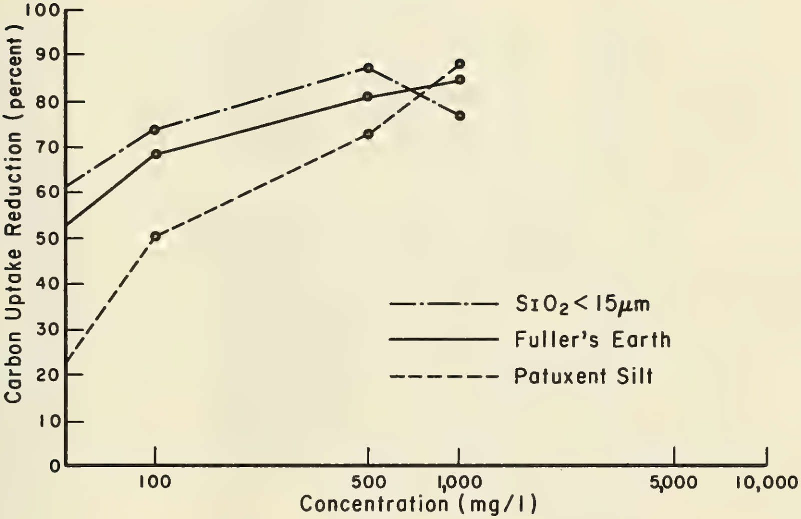

Figure 12. Reduction in maximum ingestion rate of adult *Acartia tonsa* feeding on radiocarbon-labeled *Monochrysis lutheri* at increasing concentrations of Patuxent River silt, <15 micrometers SiO_2, and Fuller's earth.

Table 2. Effect of increasing concentration of different sediment types on uptake of radioactively labeled *Monochrysis lutheri* by *Acartia tonsa*.

Concentration (milligrams per liter)	Natural sediment		Fuller's earth		<15 micrometers SiO_2	
	$\bar{X}$ ± S (N)	Reduction (percent of control)	$\bar{X}$ ± S (N)	Reduction (percent of control)	$\bar{X}$ ± S (N)	Reduction (percent of control)
Run 1						
0	12.50 ± 5.85 (7)	0.0	21.64 ± 9.28 (17)	0.0	21.64 ± 9.28 (17)	0.0
50	9.68 ± 8.00 (8)	22.6^{1}	10.03 ± 7.25 (10)	53.6^{2}	8.40 ± 4.63 (8)	61.2^{2}
100	6.15 ± 3.92 (6)	50.8^{1}	6.74 ± 2.23 (9)	68.9^{2}	5.56 ± 2.52 (8)	74.3^{2}
500	3.39 ± 1.75 (7)	72.9^{3}	4.02 ± 1.05 (7)	81.4^{2}	2.84 ± 1.21 (8)	86.9^{2}
1,000	1.49 ± 1.22 (6)	88.1^{2}	3.28 ± 2.04 (10)	84.8^{2}	4.97 ± 2.11 (8)	77.0^{2}
Run 2						
0			69.72 ± 47.53 (20)	0.0	69.72 ± 47.53 (20)	0.0
50			30.08 ± 15.83 (10)	56.9^{2}	49.96 ± 31.45 (9)	28.3^{1}
100			23.75 ± 8.82 (8)	66.0^{2}	28.72 ± 30.59 (8)	58.8^{2}
500			11.76 ± 6.75 (8)	83.1^{2}	30.24 ± 19.35 (8)	56.6^{2}
1,000			13.80 ± 9.32 (8)	80.2^{2}	18.07 ± 16.75 (9)	74.1^{2}

1. Not significant.
2. $p<0.01$.
3. $p<0.05$.

NOTE: Mean values are ~~cycles~~ counts per minute taken up in 5 minutes.

On the other hand, *A. tonsa* typically occurs in the open and less turbid waters in the Chesapeake Bay system. Apparently, this species is not stimulated to increase feeding activity by low particle concentrations which may be uncharacteristic of its natural habitat (Herman, Mihursky, and McErlean, 1968). Because these species are nonselective suspension feeders, the much reduced uptake of radioactive phytoplankton observed with increasing concentrations of suspended solids can be accounted for simply by the ingestion of increasing numbers of unlabeled particles, since the gut of both species was full during all experimental treatments.

Our results are not directly comparable with those of Wilson (1973). The plastic beads he fed to *A. tonsa* were optically sized, and particle uptake was measured according to size-frequency distribution. The median size (diameter) of food (*M. lutheri*) particles used in our experiments was 8 to 10 micrometers (optically measured). This size is below the minimum bead size (13.9 micrometers) which Wilson found that *A. tonsa* ingested. However, as Wilson noted, the artificiality of his feeding study with plastic beads may have eliminated the variables of particle shape and palatability.

Wilson (1973) demonstrated that the passive filtering behavior of *A. tonsa* could not account for the selection of a narrow, variable size range of plastic beads. However, passive filtering did exist because the minimum selected food size did not change with body size. This is typical of a process in which particles are not grasped individually, but are concentrated against closely spaced setae and are handled collectively. Bead sizes outside the selected range were ingested in the same proportion as they were present in the water column. Therefore, both selective grasping and nonselective filtering (taking all particles indiscriminately) were operating at the same time.

The median sizes (Stokes' diameters) of sediments used are reported by weight and not by optical size-frequency distributions. These were: SiO_2 (17 micrometers), <15 micrometers SiO_2 (6.2 micrometers), natural sediment (<0.8 micrometer), and Fuller's earth (<0.5 micrometer). These diameters are equivalent to those of a sphere with the same specific gravity which has settled through a column of water at some specified temperature. Since the Fuller's earth particles and those of the natural sediment were not spherical, there may have been a significant departure between values given as equivalent diameter (Stokes) and actual dimensions. In fact, the natural particle-size distribution of the Patuxent River sediment was destroyed before size analysis by the analytical procedure required to remove the large quantity of organic matter (approximate specific gravity 1.1) from the inorganic mineral particles (approximate specific gravity 2.6). In addition, both the natural sediments and the Fuller's earth formed fairly large agglomerates when suspended in saline water (microscopic observation).

The equivalent median diameters of the natural sediment (minerals) and the Fuller's earth could not be verified by the light microscope, since many of the particles were beyond the detectable size limits of that instrument. However, the particles of SiO_2 and Fuller's earth were subjected to particle population analyses using a Coulter Model T counter equipped with a 100-micrometer orifice. This counter verified our observations with the light microscope.

The larger proportion of numbers of particles was present for both of these materials at Coulter volume sizes less than 8 micrometers, even though their median equivalent spherical diameter sizes (by weight) were different by a factor of more than 30 (<0.5 micrometer for Fuller's earth versus 17 micrometers for SiO_2).

We have no evidence to show that selective grasping of particles was or was not occurring. However, reduced ingestion of food cells with increasing sediment concentrations, as demonstrated, supports the hypothesis that nonselective filtering occurred in our experiments.

c. Effects of Patuxent Silt (500 milligrams 1^{-1}) on Feeding Activity With Increasing Time. The short-term feeding experiments for *A. tonsa* and *E. affinis* (5 and 10 minutes, respectively) were extended to 125 minutes (Table 3) and 240 minutes (Table 4) for exposure to 500 milligrams 1^{-1} Patuxent River silt.

The decrease in the number of radioactive cells ingested during exposure to the sediment treatment stayed constant for as long as 125 minutes. This reduction (77.5 percent) approximates the reduction in carbon uptake in Table 2 for 500 milligrams 1^{-1} natural sediment (72 percent). The decrease in cell numbers filtered in both control and sediment treatment over the time of this experiment may indicate that cell concentration was becoming low enough to be a limiting factor.

The feeding activity pattern of *E. affinis* (Table 4) under the 500 milligrams 1^{-1} sediment treatment is more clearly defined over the 4-hour feeding period, excluding the serious errors of excretion and respiration of the carbon tracer (Conover and Francis, 1973). The control feeding activity peaked at 5, 45, and 120 minutes. Feeding activity with 500 milligrams 1^{-1} silt added was more erratic than the control pattern but generally follows it and remains significantly depressed. An increase in activity at 3.75 hours was tested and was not significant.

These data for sediment effects for exposures up to 4 hours lend support to our contention that these two species do not discriminate food from nonfood. The sediment treatment response may be indicative of the diluting effect of sediment on radioactive cell concentrations. Since the guts of these organisms were full even during the 4-hour

Table 3. Comparisons of feeding rates for *Acartia tonsa* (adults, mixed sexes) feeding on labeled *Monochrysis lutheri* cells in clean water (control) and 500 milligrams l^{-1} Patuxent River silt at increasing time intervals.

Time (minutes)	Reduction (percent of control)	Feeding rate (cells org^{-1} day^{-1})					
		Control			Patuxent silt		
		$\bar{X}$	± S.E.$_{\bar{x}}$	N	$\bar{X}$	± S.E.$_{\bar{x}}$	N
5	76.8[1]	262,801	± 71,939	(9)	60,906	± 9,580	(9)
25	85.2[2]	152,200	± 25,263	(9)	22,522	± 4,635	(10)
125	77.5[2]	140,400	± 24,741	(9)	31,644	± 8,176	(10)

1. $p<0.05$.
2. $p<0.01$.

Table 4. Comparisons of feeding rates for *Eurytemora affinis* (adults, mixed sexes) feeding on labeled *Monochrysis lutheri* cells in clean water (control) and 500 milligrams 1^{-1} Patuxent River silt at increasing time intervals.

Time (hours)	Reduction (percent of control)	Feeding rate (cells org^{-1} day^{-1})					
		Control			Patuxent silt		
		$\bar{X}$	± S.E.$_{\bar{x}}$	N	$\bar{X}$	± S.E.$_{\bar{x}}$	N
0.083	97.5[1]	3,510,179	± 456,520	(10)	87,896	± 13,654	(8)
0.167	87.3[2]	1,125,181	± 350,339	(8)	143,447	± 30,666	(8)
0.250	80.2[3]	388,312	± 104,289	(9)	76,840	± 16,854	(9)
0.50	56.7[3]	115,480	± 16,815	(9)	50,007	± 7,445	(9)
0.75	84.5[1]	473,169	± 51,393	(9)	73,561	± 23,681	(6)
1.00	70.9[2]	203,288	± 55,791	(9)	59,242	± 15,523	(8)
1.25	73.7[1]	224,669	± 33,631	(10)	59,004	± 9,488	(8)
1.50	73.2[3]	268,712	± 52,840	(10)	72,072	± 7,572	(10)
1.75	83.6[3]	281,963	± 50,613	(10)	46,269	± 15,460	(8)
2.00	94.9[1]	391,987	± 54,845	(9)	20,117	± 5,037	(8)
2.25	86.3[1]	177,508	± 20,268	(8)	24,346	± 3,802	(8)
2.50	77.5[1]	131,906	± 21,771	(8)	29,621	± 5,845	(10)
2.75	81.4[1]	104,567	± 13,363	(10)	19,404	± 3,643	(8)
3.00	85.2[1]	91,260	± 17,539	(10)	13,464	± 2,820	(10)
3.25	54.1[3]	103,008	± 12,695	(10)	47,282	± 8,649	(7)
3.50	83.7[3]	98,442	± 21,270	(10)	16,014	± 3,564	(9)
3.75	59.0[4]	126,616	± 34,299	(9)	51,924	± 4,910	(6)
4.00	57.3[3]	149,891	± 19,933	(8)	64,041	± 6,288	(10)

1. $p<0.001$.
2. $p<0.05$.
3. $p<0.01$.
4. Not significant.

feeding interval for *E. affinis* and the 125-minute interval for *A. tonsa*, we believe that the reduced ingestion of cells (reduced cell filtering rate) was caused by the high numbers of sediment particles which filled the gut and not by any reduction in the water filtering rate by the copepods.

Reductions (approximately 50 to 60 percent) in numbers of cells ingested by zooplankton in the presence of suspended nonfood particles at concentrations which are typical of "high" normal background level in Galveston Bay (Masch and Espey, 1967), storm agitation in the Chesapeake Bay, or during dredging and disposal activities, have important biological implications. The effects of a 50- to 80-percent decrease in energy flow from primary production to the secondary production level would have adverse effects on the nutrition and reproduction of zooplankton standing stocks as these are the food supply of the larval and juvenile stages of many important estuarine vertebrates and invertebrates. In addition, the extreme sensitivity of these juvenile stages to suspended solids has been demonstrated.

Supporting evidence for these implications is provided by the work of Paffenhöfer (1972) who reported serious problems of nutrition, survival, and reproduction when *Calanus helgolandicus* was exposed to suspensions of "red mud" (the residue, mostly iron oxide, after the extraction of aluminum from bauxite). Mortality of *C. helgolandicus* juveniles (copepodite) was not high (9.6 to 16 percent) even though they ingested these particles. However, the copepods were weakened and were not able to make their characteristic sudden escape movements because of reduced ingestion of phytoplankton cells when red mud was present. They ingested large quantities of particles, but did not obtain sufficient nutrient material to develop as well as did the control organisms. There was a reduced growth rate and lower weight in copepodite stages exposed to red mud. Ovary development in females was much reduced because of insufficient energy reserves. Energy reserves are stored as fat droplets in the female. None of the females exposed to red mud had fat droplets but most of the control females did (Paffenhöfer, 1972).

These results and those of Paffenhöfer do not lend support to Wilson's (1973) contention that selective feeding predominates in *A. tonsa*. Most probably nonselective feeding, i.e., taking all particles indiscriminately, does exist at high particle densities.

III. SUMMARY

Questions about the effects of suspended sediments on estuarine organisms arise when dredging is planned and environmental impact statements are prepared. It is difficult to assess the effects of particle size and concentration of suspended solids on estuarine organisms independent of, and in addition to, complicating factors associated with natural sediments such as sorbed toxic metals and pesticides, high BOD, and high nutrient content. The objective in this study was to identify the biological effects of particulate mineral solids similar in size distribution to sediments likely to be found in estuarine systems but free of complicating factors, and to study the effects of natural sediments. This report presents the effects of suspended particles on carbon assimilation of four common phytoplankton species and on the feeding rates of two common copepods.

Experimental concentrations are typical of those found in estuarine systems during flooding, storm agitation, dredging, and dredged-material disposal.

Fine silica sand was added to cultures of the common phytoplankters *M. lutheri*, *Chlorella* sp., *Nannochloris* sp., and *Stichococcus* sp., consequently reducing their carbon assimilation by 50 to 90 percent, probably by light attenuation.

Suspensions of Fuller's earth, fine silica sand, or natural sediment caused reductions in the feeding rates of the two copepods tested. Concentrations greater than 250 milligrams l^{-1} caused reductions in the ingestion of radioactively tagged *M. lutheri* by the copepods *E. affinis* and *A. tonsa*. Evidently, nonselective feeding, i.e., taking up all particles indiscriminately, does exist at high particle densities in these copepods. This results in reduced consumption of food particles which, if continued over a long enough period, could cause a serious break in the food chain. However, these experiments were designed to measure feeding rate and were not designed to measure stress on the copepods. Therefore, they cannot be used to predict the exposure time required to cause serious population depletion.

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APPENDIX

ANALYSES OF SEDIMENTS

I. INTRODUCTION

Experimental work during Project Years I and II used artificial (commercially available) mineral solids to provide base-line data for biological effects of (a) concentrations of solids, (b) different particle-size distributions, and (c) different mineral types of solids.

Work during Year III concentrated heavily on the biological effects of naturally occurring sedimentary material which was collected by anchor dredge at Long Point (38°29'30"N., 76°39'45"W.) in the Patuxent River, Maryland. This material was stored in large polyethylene tanks prior to use in our experiments. The sediment surface was covered with a layer of water (salinity range 4 to 6 parts per thousand) to maintain natural ionic equilibria between the sediment and water, as would naturally occur in the Patuxent River. A micro-oxidized sediment layer developed at the sediment-water interface in these tanks after a few days of storage.

This appendix contains the results of analyses which were performed on both the mineral (commercially available) solids and the naturally occurring sediments. Sediment characteristics measured were organic matter content (weight loss on ignition), inorganically bound heavy metals (atomic absorption analysis), and particle-size distributions (settling diameter analysis).

The particle-size distributions presented in this appendix were determined in distilled water, and may represent the basic or fundamental unit particles which can form aggregates with other units and be strongly bound by molecular and atomic forces. These composite units are stable under dispersion methods. Also, the basic particles may form agglomerates in saline water. These composites are relatively weakly bonded by electrostatic forces, surface tension, and "sticky" organic matter.

II. MATERIALS AND METHODS

1. Size Distribution.

Artificial sediments (mineral solids) used in this study were Fuller's earth (Fisher No. F-90) and silica (SiO_2, Fisher No. S-135).

Particle-size distributions of these materials were determined by the sedimentation method (American Society for Testing Materials, 1968) for paper coating clays. In addition, a finer particle-size distribution of SiO_2 was generated from the commercially available Fisher No. S-135 by allowing these solids to settle for 25 minutes through a specified distance in a column of distilled water at 20° Celsius. The solids finer by weight than 15 micrometers were calculated to be remaining in suspension in this column of water from tables presented by Trask (1968) and from Casagrande's nomographic solution of Stokes' Law given in American Society for Testing Materials (1968). The suspended particles were decanted, oven-dried for 24 hours at 100° Celsius, ground fine with a porcelain mortar and pestle, and analyzed for size distribution by the American Society for Testing Materials (1968) method performed for the other mineral solids. This particular size distribution of SiO_2 particles (median size 6.2 micrometers) is referred to in this report as <15 micrometers SiO_2 (actual analysis shows about 5 percent of the particles are >15 micrometers) (Fig. A-1, Table A-1).

The natural sediments collected from the Patuxent River were analyzed by a procedure slightly modified from the above method. Preliminary work showed that this material was approximately 75- to 80-percent salt and water by weight. Appropriate triplicate volumes of this natural material were removed from the holding tanks. These volumes were calculated to contain between 5- and 10-gram dry solids (inorganic). Also, these volumes were corrected upward for the amount (weight) of organic matter present (see method of analysis below). These quantities of solids were placed into large Pyrex beakers (1-liter capacity) and an appropriate amount of 30 percent hydrogen peroxide (H_2O_2) was added to each beaker. The amount (volume) of H_2O_2 (30 percent) needed to oxidize the organic matter present in the sediment was found to be a volume which would produce a final concentration of H_2O_2 in the sediment volume of approximately 5 percent. The oxidation reaction was quite violent initially. The reaction was allowed to proceed overnight in a hood with air bubbling slowly through the sediment-H_2O_2 mixture to remove the excess H_2O_2.

The next day, when gas evolution had ceased, 750 milliliters of deionized glass-distilled water were added to each beaker. The sediment was resuspended by stirring with a glass rod, and allowed to settle. The supernatant was decanted very carefully, and another 750 milliliters of deionized glass-distilled water rinse were added to each beaker.

A 0.2-milliliter sample of supernatant water was then taken from each beaker and the dissolved ion concentration of each solution was determined with the freezing point depression osmometer normally used in our hematological analyses. Salt concentration was read from a standard curve relating freezing point depression and osmolal concentration to sodium chloride (NaCl) concentration in mg kg^{-1} water. If the salt concentration was greater than 300 milligrams NaCl kg^{-1} water, the suspension was allowed to settle, the clear supernatant was decanted, and an additional rinse of 750-milliliter deionized

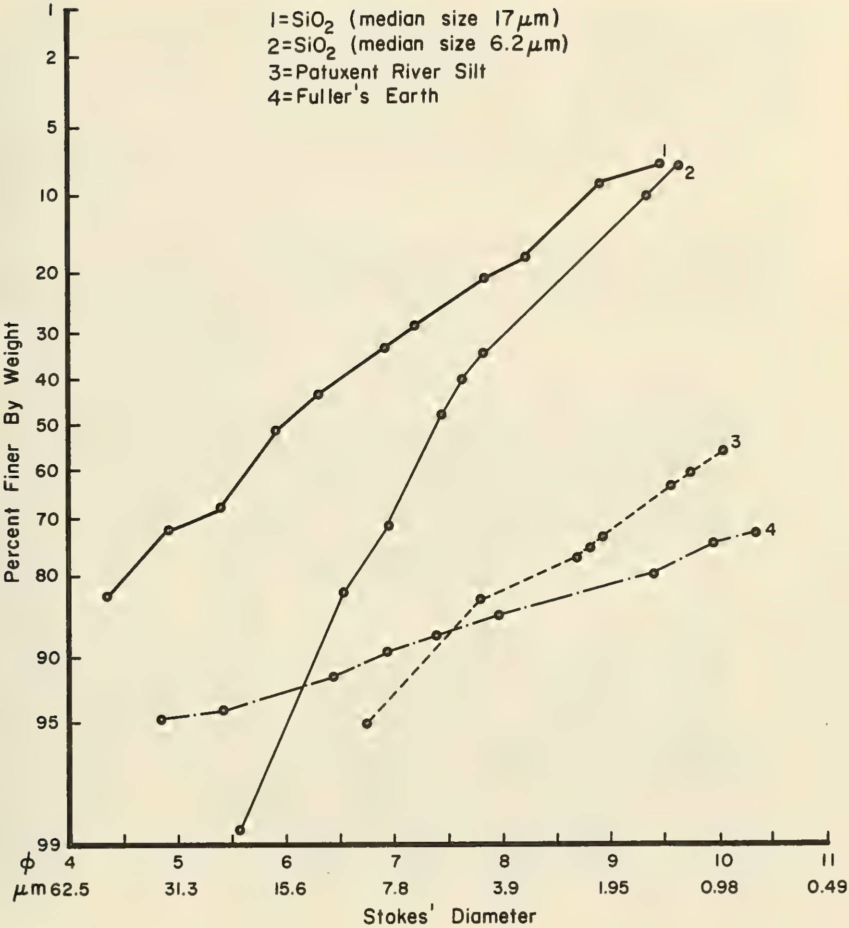

Figure A-1. Particle-size distribution of sediments.

Table A-1. Particle-size distributions of artificial sediments used in this project.

Fuller's earth (4)		SiO_2 (median size 17 micrometers) (1)		SiO_2 (median size 6.2 micrometers) (2)	
percent finer	D	percent finer	D	percent finer	D
95.0	35.8	82.2	51.5	100.0	31.8
94.2	25.3	69.9	36.9	98.7	22.6
92.0	12.7	66.1	26.2	82.0	11.5
90.0	8.1	50.9	16.9	72.0	8.5
88.3	6.4	42.4	13.4	48.0	6.0
85.8	4.0	32.2	8.6	40.0	5.2
80.0	1.6	26.1	6.8	34.7	4.5
75.0	1.0	19.1	4.3	10.7	1.6
73.3	0.8	15.3	3.5	8.0	1.4
----	----	7.2	2.2	----	----
----	----	5.1	1.5	----	----

NOTE: Percent finer = fraction (expressed as percent) finer by weight than Stokes' diameter D in micrometers. (Numbers in parenthesis refer to lines in Fig. A-1.)

distilled water was added to each beaker. The sediment was resuspended and allowed to settle. The clear supernatant was decanted and the beaker containing the washed sediment made up to 500 milliliters with fresh, deionized glass-distilled water was placed into an ultrasonic bath (45 Kilohertz) for 30 minutes. Then, the suspension was placed into a glass cylinder, made up to volume with deionized distilled water, and the analysis followed as described in American Society for Testing Materials (1968), except that the dispersing agent ($Na_4P_2O_7$) was not added.

Values are reported as percent by weight remaining in suspension (percent finer than) plotted against equivalent spherical diameters according to Stokes' Law.

2. Organic Matter Content.

Samples of the natural sediment collected from the Patuxent River at Long Point were oven-dried for 24 hours at 100° Celsius, ground fine with a porcelain mortar and pestle, and ashed for 3 hours at 500° Celsius. Organic matter values are reported as percent loss of dry weight on ignition. There was no appreciable loss of inorganic carbonate during the ashing procedure as evidenced by nonsignificant weight losses of calcium carbonate ($CaCO_3$) samples which were ashed along with the oven-dried natural sediments.

3. Heavy Metals.

Amounts of extractable cations in the mineral solids and the natural sediment samples were determined with mild acid extraction and atomic absorption analysis by Mr. David Boon, Seafood Processing Laboratory, Crisfield, Maryland. Routine procedures for inorganically bound cations as described by Perkin-Elmer Corporation (1971) and Soil Testing and Plant Analysis Laboratory (1970) were conducted for zinc, copper, iron, manganese, lead, cobalt, nickel, chromium and cadmium. Mercury values reported are for total mercury from sediments digested for 1 minute in boiling aqua regia (Dow Method, CAS-AM-70.13, 22 June 1970, revised, Chlorine Institute, Madison Avenue, New York, New York). Metal values are reported as mg kg^{-1} dry weight of solids.

III. RESULTS AND DISCUSSION

1. Size Distributions.

Particle-size distributions of the extremely fine mineral solids and natural sediment used in this project are presented in Figure A-1 and Table A-1. Useful descriptions of these materials ranked coarsest to finest by median size are as follows: Silicon dioxide (Fisher No. S-153), median size = 17 micrometers, <2 micrometers = 6 percent; Silicon dioxide (all particles <15 micrometers, median size = 6.2

micrometers, <2 micrometers = 13 percent; Patuxent River silt (composite less organic matter fraction 11.5 percent of dry weight), median size = <0.8 micrometers, <2 micrometers = 72 percent; Fuller's earth, montmorillonite and attapulgite (Fisher No. F-90), median size = <0.5 micrometers, <2 micrometers = 82 percent.

These data have been presented in such a way that graphic solutions (Folk, 1974) and mathematical calculations (Trask, 1968) can be used to determine the second, third, and fourth moments of these distributions.

Additional size-distribution analyses for the natural sediments (by date of collection) are presented in Figure A-2 and Table A-2. Median sizes ranged from a high of approximately 1.1 to a low of <0.5 micrometer (August collection). Fraction by weight finer than 2 micrometers ranged from a high of approximately 82 percent to a low of 65 percent (August collection). These particle-size distributions of solids used in our work (Tables A-1 and A-2, Figs. A-1 and A-2) are comparable with those reported by May (1973) in the mudflow from a shell dredge (Table A-3).

2. Organic Matter Content.

Organic matter content of natural sediment samples tended to increase throughout the summer of 1973 from a low of 8.9 percent in June to values in excess of 11 percent in August and September (Table A-4). A comparison of mean organic matter values (Table A-5) showed that these differences between earlier and later samples were significant. These differences may indicate significant importation of organic matter, which has settled out at Long Point, from marshes which line the shores of the Patuxent watershed. These organic matter values are as high as those reported by Masch and Espey (1967) for Galveston Bay.

Organic matter analyses were conducted on the mineral solids. No significant weight loss from ashing was detectable in the SiO_2 and Fuller's earth solids.

3. Heavy Metals.

The mineral solids contained metal amounts that were considered biologically insignificant (Table A-6). The values reported for Patuxent silt (collected at Long Point) are in the "natural" range of metal amounts found in similar estuarine salinity ranges by Huggett (Virginia Institute of Marine Science, 1973, personal communication) in the York, James, and Elizabeth Rivers which drain into the Virginia part of the Chesapeake Bay system.

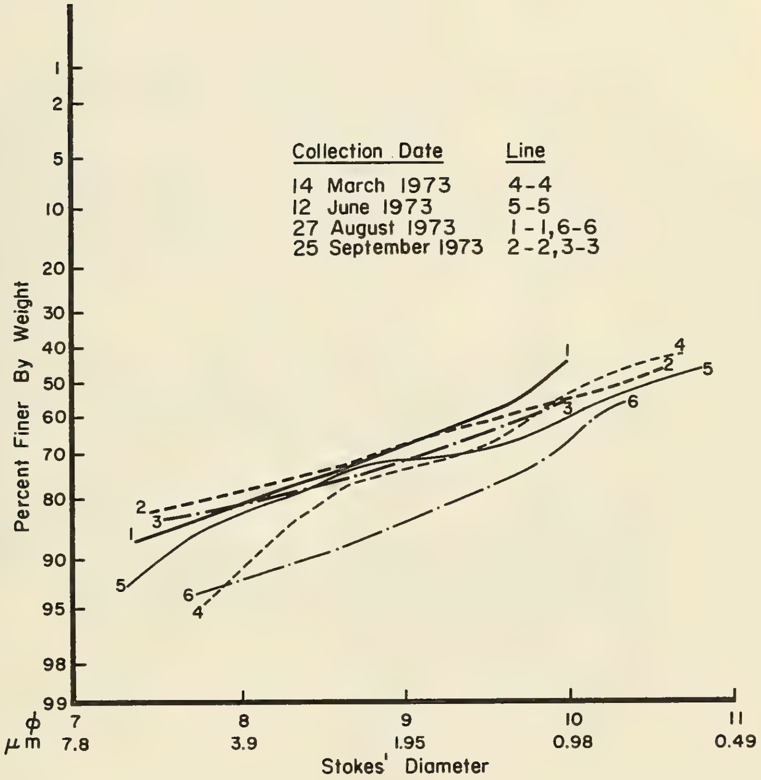

Figure A-2. Particle-size distributions of natural Patuxent River silt samples (two replicate determinations) collected by anchor dredge at Long Point.

Table A-2. Particle-size distributions of representative natural Patuxent River sediment samples collected at Long Point during 1973.

14 March		12 June		27 August				28 September			
(4-4)		(5-5)		(6-6)		(1-1)		(3-3)		(2-2)	
percent finer	D	percent finer	D	percent finer	D	percent finer	D	percent finer	D	percent finer	D
95.0	4.8	92.5	6.5	93.9	4.9	87.2	6.5	83.8	4.4	80.4	4.5
86.3	3.4	84.0	4.6	89.8	2.9	74.3	2.8	77.5	2.4	75.3	3.1
80.0	2.8	79.5	3.3	77.6	1.5	58.5	1.4	74.9	2.1	71.5	2.6
77.5	2.6	74.3	2.5	75.5	1.3	54.3	1.2	73.0	2.0	68.2	2.2
70.0	1.5	69.0	1.4	73.5	1.2	45.9	1.0	63.0	1.4	51.0	0.8
65.0	1.3	67.0	1.3	67.4	1.0	----	---	60.1	1.2	47.9	0.7
62.5	1.2	64.5	1.2	61.2	0.9	----	---	55.5	1.0	----	---
55.0	1.0	61.3	1.0	57.1	0.8	----	---	----	---	----	---
51.3	0.9	59.3	0.9	----	---	----	---	----	---	----	---
43.8	0.7	54.8	0.8	----	---	----	---	----	---	----	---
----	---	47.4	0.6	----	---	----	---	----	---	----	---

NOTE: Percent finer = fraction (expressed as percent) finer by weight than Stokes' diameter D in micrometers. (Numbers in parenthesis refer to lines in Fig. A-2.)

Table A-3. Particle-size percentage by weight of suspended solids in the mudflow from a shell dredge[1].

Meters from discharge	Size range (micrometers)				
	62-39 (percent)	38-19 (percent)	18-10 (percent)	9-5 (percent)	4-2 (percent)
0.0	10.5	20.3	25.5	27.7	16.1
15.25	11.7	26.5	25.1	25.6	11.3
30.5	8.6	21.2	30.1	32.0	7.9
122.0	4.7	17.6	24.9	29.3	23.5
244.0	0.9	14.5	27.2	34.2	23.0

1. From May, 1973.

Table A-4. Organic matter content of natural mud collected by anchor dredge from the Patuxent River (Long Point).

Collection date 1973	Sample No.	Organic content $\overline{X} \pm s$	S.E. $\overline{x}$
17 June	1	9.4120 ± 1.0038	0.3174
	2	10.3580 ± 1.0272	0.4594
	3	10.9400 ± 0.3270	0.1463
28 June	1	9.8740 ± 0.3808	0.1703
	2	9.4720 ± 0.4127	0.1846
	3	8.9120 ± 0.6079	0.2719
14 July	1	11.2467 ± 0.5555	0.2268
	2	10.0983 ± 0.8682	0.3545
27 August	1	11.4483 ± 0.8321	0.3397
	2	11.4617 ± 0.7849	0.3205
	3	11.9700 ± 0.6712	0.2740
	4	12.6483 ± 0.4317	0.1762
18 September	1	11.8567 ± 0.3038	0.1240
25 September	1	11.4217 ± 0.4881	0.1993
	2	11.8750 ± 0.5131	0.2095
	3	11.2200 ± 0.4626	0.1889

NOTE: Samples were dried for 24 hours at 100° Celsius, ground fine with a mortar and pestle, then ashed for 3 hours at 500° Celsius. Organic matter values reported are percent loss of dry weight on ignition.

Table A-5. Comparison of means of organic matter determinations by collection dates.

Sample collection dates 1973	Probability that sample means differ from previous means, 1973				
	27 June	14 July	27 Aug.	18 Sept.	25 Sept.
17 June	---[1]	---[1]	$p<0.001$	$p<0.001$	$p<0.001$
28 June	---	$P<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
14 July	---	---	$p<0.001$	---[1]	---[1]
27 Aug.	---	---	---	---[1]	---[1]
18 Sept.	---	---	---	---	---[1]

[1] Not significant.

Table A-6. Inorganically bound cations in artificial and natural sediment.

Element	Silicon dioxide	Fuller's earth	Patuxent silt (N = 13)
Zn	0.08	0.05	36.0 ± 2.0
Cu	0.04	<0.01	4.4 ± 0.4
Fe	2.6	<0.3	2100.0 ± 94.0
Mn	<0.06	0.14	2300.0 ± 260.0
Pb	<0.5	<0.5	<10.0
Co	<0.1	<0.1	< 4.0
Ni	<0.3	<0.3	< 4.0
Cd	---	---	1.0
Cr	<0.3	<0.3	< 3.0
Hg	<0.01	<0.01	< 0.2

NOTE: Extraction by 0.075 normal hydrochloric-sulfuric acid (N $HCl-H_2SO_4$) and analysis by atomic absorption spectroscopy. Values are mg kg^{-1}.

Sherk, J.A.
Effects of suspended solids on selected estuarine plankton / by J.A. Sherk, Jr., J.M. O'Connor ... [et al.]. – Fort Belvoir, Va. : U.S. Coastal Engineering Research Center, 1976.
50 p. : ill. (Miscellaneous report – Coastal Engineering Research Center ; no. 76-1) (Contract – Coastal Engineering Research Center ; DACW72-71-C-0003)
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1. Marine biology. 2. Plankton. 3. Estuarine ecology. 3. Suspended sediments. I. Title. II. O'Connor, J.M., joint author. III. Series : IV. Series : U.S. Coastal Engineering Research Center. Contract DACW72-71-C-0003.

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